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PROTEOLYTIC HYDROLYSIS OF RICE (ORYZA SATIVA BASMATI)

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PROTEOLYTIC HYDROLYSIS OF RICE (*ORYZA SATIVA* BASMATI)

By

LISA ONG GAIK AI

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

August 2001

Dedicated to:

My beloved and dearest

Mum and Dad

Sisters and Brother

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: Suraini Abd Aziz, Ph.D.

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Protein hydrolysate has been produced a long time ago. It is normally use as animal feed or as a substitute for dietary patient and the protein used is usually obtained from seafood. In this study, we are more interested in the production of protein hydrolysate from rice. The rice that was used in this study were obtained form BERNAS, Padi National Bhd, and it was pre-treated by first grinding it into fine powder, prior to analysis. The rice flour was found to consist of 11.77% of moisture, 0.46% of ash and 5.79% of crude protein. The commercial enzyme that was used for this study were Flavourzyme, Alcalase (Novo Nordisk, Denmark) and Papain (Sigma, USA). The enzyme activities and the stability of the enzymes were determined first before they were used for the production of protein hydrolysate. From the enzyme decay experiment, three of the enzymes were found to be stable at 55°C. Flavourzyme was totally denatured at 100°C, but for Alcalase (0.624%) and Papain (2.104%), some activity was still detected. Therefore, Alcalase and Papain can be clarified as being thermostable. Papain was used as comparison with Flavourzyme and Alcalase, to determine which enzyme performed better in producing the hydrolysates. From the correlation

between the free amino group, Flavourzyme (R^2 value is 0.9291) and Alcalase (R^2 value is 0.6719) were found to have a better correlation and they give a higher degree of hydrolysis compared with Papain (R^2 value is 0.5152). Therefore, Flavourzyme and Alcalase were selected for further study. The protein hydrolysate that was produced from Flavourzyme and Alcalase gave lower molecular weight subunits, which was 342 Da and 161.46 Da, respectively as detected by size exclusion high performance liquid chromatography. The free amino acids that were present in the hydrolysate were hydrolysed by the commercial enzymes and detected using the PICO-Tag method. From the kinetic analysis, Alcalase was found to form enzyme-substrate complex easier compared to Flavourzyme. However, the production rate for Alcalase was lower compared with Flavourzyme, which was 8.14 for Alcalase and 34.80 for Flavourzyme using Eadie-Hofstee equation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

HIDROLISIS PROTEOLITIK KE ATAS BERAS (*ORYZA SATIVA* BASMATI)

Oleh

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Ogos 2001

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Hidrolisat protein telah dihasilkan beberapa dekad yang lepas. Ia biasa digunakan sebagai makanan haiwan ternakan atau makanan gantian untuk pesakit yang sering diperolehi daripada hasilan lautan. Dalam kajian ini, penghasilan hidrolisat protein daripada tepung beras diutamakan. Beras diperolehi daripada BERNAS, Padiberas National Bhd. dan pre-rawatan telah dilakukan ke atas beras untuk dijadikan tepung yang halus dengan mengisar beras itu. Selepas itu, kandungan tepung beras dianalisa. Ia mengandungi 11.77% air. 0.46% abu dan 5.79% protein kasar. Aktiviti enzim and kestabilan enzim terhadap suhu ditentukan terlebih dahulu sebelum ia digunakan untuk penghasilan hidrolisat protin. Ketiga-tiga enzim didapati paling stabil pada suhu 55°C. Flavourzyme dinyahaslikan secara keseluruhannya pada suhu 100°C, manakala Alcalase (0.624%) dan Papain (2.104%) masih lagi menunjukkan aktiviti yang agak lemah. Ini menunjukkan bahawa Alcalase and Papain termasuk dalam golongan enzim yang stabil pada suhu tinggi. Papain digunakan sebagai perbandingan dengan Flavourzyme dan Alcalase untuk melihat keberkesanan penghasilan protein hidrolisat dalam kajian ini. Daripada keputusan yang diperolehi, Flavourzyme

(nilai R^2 ialah 0.9291) and Alcalase (nilai R^2 ialah 0.6719) menunjukkan korelasi yang lebih baik berbanding dengan Papain (nilai R^2 ialah 0.5152). Oleh itu, Flavourzyme dan Alcalase dipilih untuk kajian yang selanjutnya. Hidrolisat protein yang dihasilkan dengan mengunakan enzim Flavourzyme dan Alcalase menghasilkan berat molekul yang kecil, iaitu 342 Da dan 161.46 Da. Acid amino bebas yang dihasilkan oleh hidrolisat dikenalpasti dengan menggunakan kaedah PICO-Tag. Analisa kinetik menunjukkan Alcalase lebih mudah membentuk komplex enzim-substrat berbanding dengan Flavourzyme, tetapi Flavourzyme lebih cepat menghasilkan produk berbanding dengan Alcalase, di mana 8.14 bagi Alcalase dan 34.80 bagi Flavourzyme dengan menggunakan persamaan Eadie-Hofstee.

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I certify that an Examination Committee met on 23rd October 2001 to conduct the final examination of Lisa Ong Gaik Ai on her Master of Science thesis entitled "Proteolytic Hydrolysis of Rice (*Oryza sativa* Basmati)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the Candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Univeristi Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

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DECLARATION FORM

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LISA ONG GAIK AI

Date: 31 Dec 2007

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LIST OF ABBREVIATIONS

AH	Amount of hydrolysis
AU	Anson Unit, a measure of proteolytic activity on denatures hemoglobin at pH 7.5 and 25°C
B	Volume of base consumed during the hydrolysis (L)
BCA	bicinchoninic acid
BSA	Bovine serum albumin
DH	Degree of hydrolysis
h	Hydrolysis equivalents, defined as equivalents of peptide bonds cleaved per kg protein
h_{tot}	Total number of peptide bonds in a protein, expressed in the same unit as h
HPA	Hide powder azure
HPLC	High performance liquid chromatography
K_m	Michaelis constant
MP	mass of protein
MSG	Monosodium glutamate
N_b	Normality of base in protein hydrolysis experiments
TNBS	Trinitrobenzensulphonic acid
UV	ultraviolet
V_{max}	Maximum velocity
α	Degree of dissociation of the α-amino group

CHAPTER 1

INTRODUCTION

Proteins are essential components of the diet for human nutrition as sources of energy and amino acids. Their nutritional quality depends on their amino acid content and on the physiological utilisation of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation (Friedman, 1996). Also availability of amino acids varies with protein source, processing treatment and interaction with other components of diets (Clemente *et al.*, 1999).

Enzymatic modification of proteins using selected proteolytic enzyme preparations to cleave specific peptide bonds is widely used in the food industry. Hydrolysis of food proteins has a long history, mainly for vegetable and milk protein; these proteins are used in the food industry.

Protein hydrolysates possess properties that make them attractive as a protein source in human nutrition. Hydrolysates are used in products for special nutrition, such as diets for elderly and patients with impaired gastrointestinal absorption, hypoallergenic infant formulas, sports nutrition and weight-control diets, as well as in consumer products for general use (Frøkjaer, 1994; Schmidl *et al.*, 1994). Peptide based formulas have been useful because of their high solubility especially under acidic conditions; even during heat treatment, the peptides remain in solution (Frøkjaer, 1994). In addition to their solubility in a wide range of pH and other functional

properties, such as improvement of texture and water binding capacity (Lin *et al.,* 1997), protein hydrolysates are physiologically better than intact proteins because their intestinal absorption appears to be more effective (Calderón de la Barca *et al.*, 2000).

It is important that the extent of hydrolysis be controlled, since too much proteolysis can decrease the benefits and reduce the functionality of the hydrolysate. More importantly, excessive hydrolysis can course bitterness in protein hydrolysates. The bitterness seems more pronounced if the hydrolysis is extensive, while limited hydrolysis prevents the bitterness and has the added benefit of removing bound odorants and off-flavours.

The protein source most commonly used in nutritional products are casein and whey proteins. However, plant proteins are finding commercial application in a number of formulated foods as an alternative to proteins from animal sources (Clemente *et al.*, 1999). Among plant proteins, soybean and wheat were the source most widely used to obtain protein hydrolysates, but other source such as peas (Periago *et al.*, 1998) and chickpeas (George *et al.*, 1997) have been successfully used.

Glutamic acid and its salts have a long history of use in food to enhance the flavour. Monosodium glutamate (MSG) is by far the most widely used glutamate. It is used at a concentration of 0.2-0.8% in a variety of foods such as soups, broth, sauces, gravies, flavouring and spice blends, and in many canned frozen meats, poultry, vegetables and combination dishes

(Hamada *et al.*, 1998). Glutamates as part of a protein are not flavour enhancers, but glutamates bound into peptide structure may have the flavour enhancing properties of the free form. Due to the relatively high content of asparagine and glutamine in rice protein, deamidated peptides and protein hydrolysates can be an excellent source of flavour enhancing ingredient for food applications (Hamada *et al.*, 1998).

The objectives of this project are

1) To pre-determine the enzymatic hydrolysis parameters for hydrolysis of rice protein,
2) To produce rice protein hydrolysate using optimum enzymatic hydrolysis paramerters,
3) To investigate the action and use of food grade proteolytic enzymes on rice protein for the production of protein hydrolysate as food ingredient.
4) To characterise the rice protein hydrolysate obtained

CHAPTER 2

LITERATURE REVIEW

2.1 Rice Protein

Basmati rice is a long grained aromatic variety of rice that is cultivated in areas of Northern India and Pakistan, mainly in Punjab. The word "Basmati" is Hindi for "the fragrant one" or "fragrant earth". Basmati is widely recognised as having specific desirable qualities.

In rice, the protein of the starchy endosperm consists of about 15% salt-soluble proteins (albumin and globulin), 5% ethanol-soluble proteins (prolamin), and 80% diluted alkali- or acid-soluble proteins (glutelin). Most of these proteins accumulate in concentrated deposits, which is the protein body. The most abundant rice storage proteins, glutelin, accounts for about 80% of the total protein in starchy endosperm. This protein has two groups of polypeptides with molecular sizes of 22-23 and 37-39 kDalton. Prolamin consists of three major polypeptides with molecular sizes of 10, 13, and 16 kDalton. They are found in the protein bodies of starchy endosperm (Masumura *et al.*, 1991).

Rice protein has a lysine content of about 4 g/16.8g of nitrogen, one of the highest among cereal proteins. Lysine is the first limiting essential amino

acid of rice protein as in other cereals. The amino acid pattern of rice is better than most cereals, this is due to most cereals is lack of the lysine. The major free amino acids in a developing rice grain include alanine, aspartic acid-asparagine, valine, glutamic acid, histidine, and ornithine, whereas the principal amino acids of rice protein are alanine, arginine, aspartic acid, glutamic acid, leucine, and valine (Lu and Luh, 1991).

2.2 Protein Hydrolysate and Its Application

Protein is a high molecular weight compound of low molecular weight amino acids and plays an important role in our diet. But the modification forms may differ in their nutritional, dietetic and functional properties and their advantages can be adopted as and when required (Dave *et al.*, 1991). One of the modified forms of protein is protein hydrolysate, which is a rich amino acid mixture derived as a result of hydrolysis of protein either by chemical or proteolytic enzymes.

Proteolytic modification of food proteins to improve palatability and storage stability of the available protein resources is an ancient technology (Adler-Nissen, 1986). Hydrolysates can be defined as proteins that are chemically or enzymatically broken down into peptides of varying sizes. Protein hydrolysates are produced for a wide variety of uses in the food industry, including milk replacers, protein supplements, stabilisers in

beverages and flavour enhancers in confectionery products (Kristinsson and Rasco, 2000).

The benefits of hydrolysing food proteins to make functional protein ingredients and nutritional supplements is a more recent technology, with the first commercially available protein hydrolysates appearing only around the late 1940s (Mahmoud *et al.*, 1992). Although production is massive worldwide, the proper control of the process and the exact mechanism behind protein hydrolysis is in most cases not fully understood. Recent advances have given researcher insight into the connection between the process/extent of hydrolysis and the physicochemical properties of the hydrolysed protein. Recent research on enzyme catalysis has also aided the proper selection of enzyme catalysts and processing conditions to obtain better control over the reaction and characteristics of the final products (Kristinsson and Rasco, 2000).

The main advantage of enzymatic hydrolysis is that the amino acid profile remains the same as the substrate protein with no destruction of amino acids. In addition, the production of enzymatic hydrolysates may be economically more favourable, especially in comparison with synthetic amino acids mixtures (Clegg, 1977).

The major disadvantage for chemical hydrolysis is the present of 3 monochloro-propane-1,2,-diol (3MCPD) which can cause cancer. 3-MCPD is a member of a group of contaminants know as Chloropropanols, which

includes know genotoxis animal carcinogens such as 1.3-dichloropropan-2-ol (Hodgson, 2001; Anonymous, 2001). 3-MCPD has been detected at low levels in many foods and food ingredients, such as breads, savory crackers, toasted biscuits, cheese, doughnut, burger, salami, malts and modified starched (Hodgson, 2001). 3-MCPD is a by product in soy sauce and in hydrolysed vegetable protein produce through acid hydrolysis. Usually present in trace amount (< 1 mg/kg) (Anonymous, 2001; Hodgson, 2001). The Joint FAO/WHO expert Committee on Food Additives (JECFA) has concluded that 3-MCPD is an undersirable contaminant in food and recommended that its concentration in hydrolysed proteins should be reduced to the lowest level technically feasible (Hodgson, 2001).

The applications of the protein hydrolysate are widely used. In the medical line, protein hydrolysate can inhibit bacterial translocation (Kops *et al.*,1997), inhibit beta-glucuronidase (Gourley *et al.*, 1997), induction of systemic immunologic torelance to beta-lactoglobulin (Fritsché *et al.*, 1997), hypoallergenic in infant formulas (Plebani *et al.*, 1997), increase the deranulation (Ju *et al.*, 1997), used as antiosidant (Ovsiannikova *et al.*, 1999) infulence the activity of some ATPase system (Ivanov *et al.*, 1999), induction of beta lactamase (O'Connor and Zusman, 1999), promoting growth and production in animal cells culture (Franek *et al.*, 2000) etc. In food industrial, protein hydrolysis produce from chick pea can produce methionine by proteases of *Bacillus amyloliquefaciens* (George *et al.*, 1997), production of seafood flavour from red hake (Imm and Lee, 1999), improve the nutritional value with the addition of protein hydrolysate (Peluzio *et al.*, 1998), flavour